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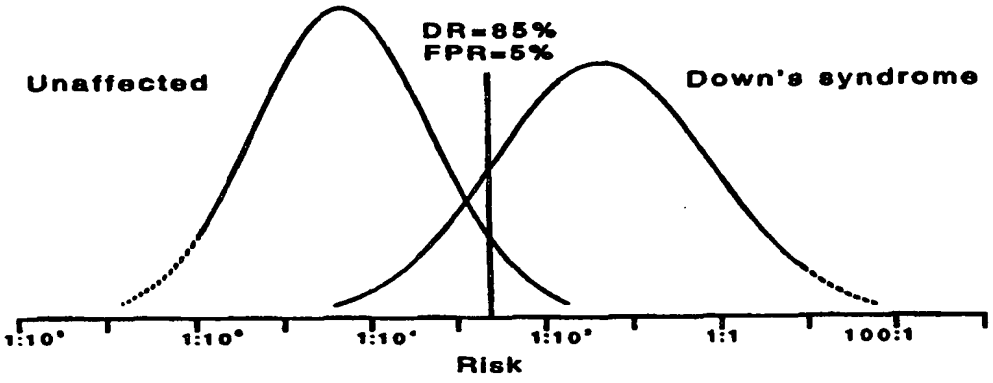

(57) Abstract

A method of screening for fetal Down's syndrome is described. Screening marker levels are measured. These may be measurements of a biochemical marker in a maternal sample or measurements of a marker from an ultrasound scan. The marker levels are used to calculate a risk of Down's syndrome. Instead of using markers from a single stage of pregnancy, the method uses markers from two or more different stages of pregnancy, typically one being in the first and another being in second trimester. The method may be automated.

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ANTENATAL SCREENING FOR DOWN'S SYNDROME

This invention relates to a method and apparatus for determining for screening purposes whether a pregnant woman is at an increased risk of fetal Down's syndrome.

The risk of Down's syndrome in a fetus is known to increase with the age of the mother. In addition, abnormally high or low concentrations of certain substances in the maternal serum (biochemical markers), and abnormally large or small measurements of certain ultrasonographic signs (ultrasound markers), are known to be associated with an increased risk of Down's syndrome in the fetus.

Information on one or more of these biochemical or ultrasound markers (collectively called screening markers) can be combined with the age-related risk of Down's syndrome, to form the basis of a screening test.

The aim of a screening test is to identify women who are at a sufficiently high risk of Down's syndrome to justify a further test which is diagnostic of Down's syndrome. Such further diagnostic tests, eg. chorionic villus sampling or amniocentesis, involve sampling procedures that carry a certain risk to the mother and/or fetus, the induction of miscarriage and fetal limb defects being among the recognised hazards. There is, therefore, a need for screening tests that maximise the chance of identifying those pregnancies at highest risk of Down's syndrome, so as to justify further diagnostic tests with their attendant risks.

The effectiveness of a screening test depends on its ability to discriminate between pregnancies with Down's syndrome and unaffected pregnancies. The discriminatory power of a test is usually specified in terms of the detection rate achieved for a given false-positive rate, or in terms of the false-positive rate required to achieve a given detection rate. The detection rate is the proportion of Down's syndrome pregnancies with a positive result. The false-positive rate is the proportion of unaffected pregnancies with a positive

result.

Different screening markers generally impart more discriminatory power to a screening test at one stage of the pregnancy than at other stages. Currently employed screening tests rely on certain combinations of biochemical and ultrasound markers that have been identified as being effective when used together at a specific, single stage of pregnancy.

For example, the "combined test" carried out in the first trimester using nuchal translucency and free β-hCG and PAPP-A as screening markers can achieve an 80% detection rate with a 5% false-positive rate. The "triple test" carried out in the second trimester uses AFP, uE_3 and hCG as screening markers. The "quadruple test" carried out in the second trimester uses the screening markers of the "triple test" plus inhibin-A. The "triple test" and the "quadruple test" can achieve an 80% detection rate with a false positive rate of 10% and 6.6%, respectively. However, a screening test with greater discriminatory power would be desirable. A high false-positive rate means that a large number of women with screen-positive results in fact have unaffected pregnancies. For these unaffected women the screen-positive result, quite apart from causing considerable anxiety, might lead to a diagnostic procedure such as amniocentesis or chorionic villus sampling which have a risk of miscarriage of about 1 in 100.

The present invention relies on screening markers obtained from two or more different stages of pregnancy. In particular, according to the first aspect of the present invention there is provided a method of determining whether a pregnant woman is at an increased risk of having a fetus with Down's syndrome, the method comprising the steps of:

measuring at least one screening marker level from a first stage of pregnancy by:

(i) assaying a sample obtained from the pregnant woman at said first stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said first stage of pregnancy;

measuring at least one screening marker level from a second stage of pregnancy by:

(i) assaying a sample obtained from the pregnant woman at said second stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said second stage of pregnancy; and

determining the risk of Down's syndrome using the measured screening marker levels from both the first and second stages of pregnancy.

The risk of Down's syndrome may be determined by a statistical analysis of the screening marker levels based on reference data which may be derived from existing or future studies. Preferably the step of determining the risk of Down's syndrome comprises deriving the likelihood ratio of Down's syndrome using a multivariate analysis based on distribution parameters derived from a set of reference data.

Such a method can provide a single integrated screening test that is more effective at identifying affected pregnancies than tests which are based on samples collected at a single stage of pregnancy, that is it yields a higher detection rate at the same false-positive rate or a lower false-positive rate at the same detection rate. For example if the risk of Down's syndrome is determined by a method integrating nuchal translucency measurement and PAPP-A in the first trimester and the "quadruple test" using AFP, uE_3 hCG and inhibin-A as markers in the second trimester, it is estimated that at a detection rate of 80%, the false-positive rate will be brought below 1%. This is a considerable improvement over the 5% false positive rate for the "combined test" alone. This means fewer unaffected pregnancies will be classified as screen-positive. Furthermore, at an 80%

detection rate, if the expense of the additional screening measurements amounts to, say, US$100 there would be no overall extra expense because the extra screening costs would be offset by savings from performing substantially fewer invasive diagnostic tests.

The present invention utilises the fact that the ability of different screening markers to discriminate between Down's syndrome pregnancies and unaffected pregnancies varies according to the stage of pregnancy. For example, the screening marker PAPP-A is most useful before 14 weeks, but not afterwards, and vice versa with the screening marker inhibin-A, as summarised in Wald NJ, Kennard A, Hackshaw A, McGuire A. (1997); Antenatal screening for Down's syndrome. J Med Screen 4,181-246.

The present invention can also provide the important advantage of permitting the use of the maternal serum AFP for screening for open neural tube defects (which is best done after 15 weeks of pregnancy) as well as using the earlier test results for Down's syndrome screening.

According to a second aspect of the prevention invention there is provided a method according to any one of preceding claims, further comprising: determining a first risk estimate of Down's syndrome using the measured screening marker levels from the first stage of pregnancy; comparing the first risk estimate with a predetermined cut-off level to initially classify the pregnant woman as screen-positive or screen-negative based on the comparison; and performing said steps of measuring at least one screening marker level from a second stage of pregnancy and determining the risk of Down's syndrome using the measured screening marker levels from both the first and second stages of pregnancy if the pregnant woman is initially classified as screen-negative.

The processing of the measurements of the screening marker levels may be implemented by a data processing system, suitably a general purpose computer executing an appropriate program. Therefore, according to a third aspect of the

present invention, there is provided an apparatus for determining whether a pregnant woman is at an increased risk of having a fetus with Down's syndrome, the apparatus comprising:

data input means for inputting a measurement of at least one screening marker level from a first stage of pregnancy obtained by:

(i) assaying a sample obtained from the pregnant woman at said first stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said first stage of pregnancy;

data input means for inputting a measurement of at least one screening marker level from a second stage of pregnancy obtained by

(i) assaying a sample obtained from the pregnant woman at said second stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said second stage of pregnancy; and

calculation means for determining the risk of Down's syndrome using the input screening marker levels from both the first and second stages of pregnancy.

According to a fourth aspect of the present invention, there is provided a computer program which when executed on a computer causes the computer to perform a process for determining a pregnant woman's risk of having a fetus with Down's syndrome, the process comprising the steps of:

receiving an input of a measurement of at least one screening marker level from a first stage of pregnancy obtained by:

(i) assaying a sample obtained from the pregnant woman at said first stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an

ultrasound scan taken at said first stage of pregnancy;

receiving an input of a measurement of at least one screening marker level from a second stage of pregnancy obtained by

(i) assaying a sample obtained from the pregnant woman at said second stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said second stage of pregnancy; and

determining the risk of Down's syndrome using the input screening marker levels from both the first and second stages of pregnancy.

To allow better understanding the following description of a method and apparatus for screening for fetal Down's syndrome according to the present invention is given by way of non-limitative example with reference to the drawings in which:

Figs. 1, 2, 3 and 4 show the distributions of risk in (a) Down's syndrome and (b) unaffected pregnancies using different sets of markers at two stages in pregnancy;

Fig. 5 is a flowchart illustrating a specific method according to the present invention, in particular, a screening test that involves deriving a risk estimate from measurements made on biochemical samples and/or ultrasound images collected at different stages of pregnancy;

Fig. 6 is a flowchart illustrating the procedure for calculating multiples of the median (MoM) for biochemical and ultrasound markers;

Fig. 7 is a flowchart illustrating the procedure for adjusting MoM values to allow for various factors, other than gestational age, that may affect biochemical marker levels;

Fig. 8 is a flowchart illustrating the procedure for selecting the appropriate parameters of the distributions of screening markers in affected and unaffected pregnancies; and

Fig. 9 is a flowchart illustrating the procedure for calculating the age-specific risk of Down's syndrome.

Measurements carried out on biochemical samples may include assaying one or more of the following biochemical markers of Down's syndrome in maternal serum or plasma, among others :-

- alpha feto-protein (AFP)
- unconjugated oestriol (uE_3)
- human chorionic gonadotrophin (hCG)
- free alpha sub-unit of hCG (free α-hCG)
- free beta sub-unit of hCG (free ß-hCG)
- inhibin , preferably dimeric inhibin-A (inhibin A)
- pregnancy-associated plasma protein A (PAPP-A)

Measurements carried out on biochemical samples may also include assaying one or more of the following biochemical markers of Down's syndrome in maternal urine, among others:-

- beta-core hCG
- total oestriol

Measurements carried out on ultrasound images may include one or more of the following ultrasound markers of Down's syndrome, among others :-

- nuchal translucency (NT) thickness, nuchal fold thickness
- femur length
- humerus length
- hyperechogenic bowel
- renal pyelectasis
- fetal heart rate
- certain cardiac abnormalities

Use of the above and other screening markers at a single stage of pregnancy is known, so the specific techniques by which measurements are obtained need not be described in detail here. In the known methods the biochemical and ultrasound markers levels are interpreted in combination with maternal age, to derive a risk estimate. The estimation of risk is conducted using standard statistical techniques. For

example, known methods are described in Wald NJ, Cuckle HS, Densem JW, et al (1988); Maternal serum screening for Down's syndrome in early pregnancy. BMJ 297, 883-887 and in Royston P, Thompson SG (1992); Model-based screening by risk with application to Down's syndrome. Stat Med 11, 256-268.

In the present method, a single risk estimate is derived from measurements of marker levels carried out on biochemical samples (eg. serum or plasma or urine or cells) and/or ultrasound images which are obtained sequentially at two or more different stages of pregnancy. Thus the calculation can be integrated as a single test at one stage. The individual measurements are obtained by using known methods. One or more screening markers from each of the stages of pregnancy may be used. Any markers which are effective at each particular stage may be selected. For example, in one embodiment of this invention, the markers from the first trimester between 8 to 13 weeks of pregnancy are the "combined test" markers (NT, free ß-hCG and PAPP-A) and the markers from the second trimester between 14 to 22 weeks are the "quadruple test" markers AFP, uE_3, total hCG and inhibin-A. Preferably, one would not use both free β-hCG from the first trimester and total hCG from the second trimester because of an expected high correlation between these markers. Therefore the preferred embodiment is to use NT and PAPP-A from the first trimester and the "quadruple test" markers from the second trimester. Other possible marker combinations are set out in Tables 4a and 4b below. In practice one might need to omit the use of NT at some test centres which are not experienced in its measurement or to omit the use of inhibin-A at some test centres which prefer to retain their current use of the "triple test" markers instead of the "quadruple test" markers.

The measured marker levels are used in combination, preferably together with maternal age, to derive a risk estimate of having an affected pregnancy.

Most screening markers levels are known to vary with gestational age. To take account of this variation each

marker level may be expressed as a multiple of the median level (MoM) for unaffected pregnancies of the same gestational age. Especially, for markers derived from ultrasound scans, crown-rump length (CRL) or biparietal diameter (BPD) measurement are alternative measures of gestational age. MoMs may be adjusted in a known way to take account of factors which are known to affect marker levels, such as maternal weight, ethnic group, diabetic status and the number of fetuses carried.

When using several markers in combination to screen for a particular disorder, it is desirable to take account of correlation between the markers. If two markers are perfectly correlated, one adds nothing to the other in assessing the risk of having the disorder, whereas if they are completely uncorrelated, each provides an independent measure of risk. To the extent that they may be partially correlated, each will provide some independent information. The correlations between markers known to be suitable for use at the same stage of pregnancy are known, for example as summarised in Table 1 below for the preferred markers.

In the present method, the markers from different stages of pregnancy are assumed to be independent of each other among affected and unaffected pregnancies. There may be some degree of correlation between these markers but this is unlikely to have a material effect on the estimated screening performances. In any case, if required, such correlation coefficients can be incorporated into the calculation of risk estimates in the same way as correlation coefficients are already used in the present method.

Calculation of risk from the measured marker levels is based on the observed relative frequency distribution of marker level in (a) Down's syndrome and (b) unaffected pregnancies. Any of the known statistical techniques may be used. Preferably the multivariate Gaussian model is used, which is appropriate where the observed distributions are reasonably Gaussian. Such multivariate Gaussian analysis is

in itself known, for example from Wald NJ, Cuckle HS, Densem JW, et al (1988) and Royston P, Thompson SG (1992) referred to above. Thus no detailed discussion is necessary, but a summary is given as follows.

If a matrix representation is used, the height H of the Gaussian distribution for a given set of measured levels is given by the equation:

$$H = \frac{1}{\prod(\sigma).(2\pi)^{p/2}.\det(\mathbf{R})^{1/2}} \exp(-1/2.\mathbf{Z}^T.\mathbf{R}^{-1}.\mathbf{Z})$$

where p is the number of markers, $\Pi(\sigma)$ is the product of the standard deviations for each distribution, $\underline{Z}$ is a matrix containing the measured level of each marker expressed in standard deviation units, namely ((measured level - mean) / standard deviation), and $\underline{R}$ is a matrix containing the correlations between the tests.

For each test two Gaussian heights are calculated, (a) one for the Down's syndrome distribution and (b) the other for the unaffected distribution. For this calculation the necessary statistical distribution parameters which specify the Gaussian distribution are the mean, standard deviation and correlations for the two distributions. These are known, being derivable from observed distributions and are given for some markers for example in Wald NJ, Hackshaw AK (1997); Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. Prenat Diagn 17,821-829; in Wald NJ, Densem JW, George L, Muttukrishna S, Knight PG (1996); Prenatal screening for Down's syndrome using inhibin-A as a serum marker. Prenat Diagn 16,143-153; and in Wald NJ, Densem JW, George L, Muttukrishna S, Knight PG (1997) Inhibin-A in Down's syndrome pregnancies: revised estimate of standard deviation. Prenat Diagn 17,285-290, as summarised in Table 1 below for the preferred markers. The distribution parameters are stored as reference data for use in the analysis.

Table 1: Standard deviations, correlation coefficients, and

means (log_{10} MoM) for unaffected and Down's syndrome pregnancies for screening markers (based on the gestational age estimate using an ultrasound scan examination, with maternal weight adjustment of serum markers).

		Unaffected pregnancies	Down's syndrome pregnancies
STANDARD DEVIATIONS			
Nuchal translucency		0.1717	0.2396
PAPP-A		0.2659	0.3471
Free β-hCG		0.2833	0.2870
AFP		0.1789	0.1821
uE_3		0.1102	0.1210
Total hCG		0.2239	0.2520
Inhibin-A		0.2154	0.1986
CORRELATION COEFFICIENTS			
Nuchal translucency	PAPP-A	0.0000	0.0000
PAPP-A	Free-β-hCG	0.1407	0.0648
Free β-hCG	Nuchal translucency	0.0000	0.0000
AFP	uE_3	0.0901	0.1770
AFP	Total hCG	0.0596	0.2148
AFP	Inhibin-A	0.0780	0.1045
uE_3	Total hCG	-0.0586	-0.0474
uE_3	Inhibin-A	0.0175	-0.1024
Total hCG	Inhibin-A	0.1882	0.2493
MEANS			
Nuchal translucency		0.0000	0.3118
PAPP-A		0.0000	-0.3704
Free β-hCG		0.0000	0.2540
AFP		0.0000	-0.1427
uE_3		0.0000	-0.1411
Total hCG		0.0000	0.3023
Inhibin-A		0.0000	0.2522

The ratio of the two Gaussian heights gives the likelihood ratio. The likelihood ratio is a measure of the increased risk of having a disorder, given a particular combination of test results, compared to the background risk

(that is, the risk in the absence of the test results).

The likelihood ratio is multiplied by the known background risk, which is preferably the age-specific risk, to calculate the improved estimate of risk. The age-specific risk can be calculated using the maternal age distribution of England and Wales for 1984-1988 (taken from Office of Population Censuses and Surveys (1985-1990); Birth Statistics, Series FM1, Nos, 11, 12, 15-17, London: HMSO) and the birth rate of Down's syndrome in live births (taken from Cuckle HS, Wald NJ, Thompson SG (1987); Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level, Br J Obstet Gynaecol 94, 387-402).

The estimated risk is classified as screen-positive or screen-negative based on a comparison with a predetermined cut-off. The value of the cut-off may be altered to vary the detection rate and false-positive rate.

Expected Down's syndrome detection rates and false-positive rates using the present invention have been estimated. They show an improved performance over the tests from a single stage of pregnancy. Tables 2, 3 and 4 illustrate this improved performance. Performance is shown in tables 2a, 3a and 4a in terms of the detection rate achieved at specified false-positive rates and in tables 2b, 3b and 4b in terms of the false-positive rate achieved at specified detection rates. The estimates are based on a gestational age estimate using an ultrasound scan, with maternal weight adjustment of serum markers. Tables 2a and 2b show the performance of different screening tests currently performed between 10 and 13 weeks of pregnancy. Tables 3a and 3b show the performance of different screening tests currently performed between 14 and 22 weeks of pregnancy. Tables 4a and 4b show the performance of four different integrated screening tests according to the present invention. Tables 5a and 5b show the performance of the preferred embodiment (using NT and PAPP-A from the first trimester and the "quadruple test"

markers from the second trimester), and also with the omission of inhibin-A, NT and both.

The performance of the integrated tests of the present method can be seen to be superior, because at each false-positive rate the detection rate of the present method is higher than that of each currently available tests based on a single stage of pregnancy, and at each detection rate the false-positive rate of the present method is lower than that of the currently available tests. As shown in Tables 5a and 5b, even omitting inhibin-A, NT or both it is of benefit to integrate the markers from the first and second trimesters into a single screening test.

Table 2a

	Detection rate (%). Maternal age with:			
False positive rate (%)	AFP and total hCG	AFP, uE_3 and total hCG	AFP, uE_3, free α-hCG and free β-hCG	AFP, uE_3, total hCG and inhibin-A
1	35	46	53	54
2	44	55	61	64
3	50	62	66	69
4	55	66	70	73
5	59	69	73	76

Table 2b

	False-positive rate (%). Maternal age with:			
Detection rate (%)	AFP and total hCG	AFP, uE_3 and total hCG	AFP, uE_3, free α-hCG and free β-hCG	AFP, uE_3, total hCG and inhibin-A
55	4.0	1.9	1.2	1.1
60	5.4	2.7	1.8	1.6
65	7.1	3.8	2.7	2.2
70	9.4	5.2	4.0	3.2
75	12	7.3	5.9	4.5
80	17	10	8.9	6.6
85	22	15	14	9.8
90	30	22	22	15
95	45	35	37	26

Table 3a

	Detection rate (%). Maternal age with:		
False positive rate (%)	NT	Free β-hCG and PAPP-A	NT, free β-hCG and PAPP-A
1	43	40	62
2	51	49	70
3	56	55	75
4	59	59	78
5	63	62	80

Table 3b

	False-positive rate (%). Maternal age with:		
Detection rate (%)	NT	Free β-hCG and PAPP-A	NT, free β-hCG and PAPP-A
55	2.8	3.1	0.5
60	4.2	4.4	0.8
65	5.9	6.2	1.3
70	8.7	8.6	2.0
75	13	12	3.1
80	18	17	5.0
85	26	23	8.1
90	37	34	14
95	58	51	27

Table 4a

	Detection rate (%). Maternal age with:			
False positive rate (%)	At 10-13 weeks: PAPP-A At 14-22 weeks: AFP, uE_3 total hCG and inhibin-A	PAPP-A and free β-hCG AFP, uE_3 and inhibin-A	NT and PAPP-A AFP, uE_3, total hCG and inhibin-A	NT, PAPP-A and free β-hCG AFP, uE_3, and inhibin-A
1	66	65	81	80
2	75	74	86	86
3	79	78	89	89
4	82	81	91	91
5	85	84	92	92

Table 4b

Detection rate (%)	False-positive rate (%). Maternal age with: At 10-13 weeks: PAPP-A At 14-22 weeks: AFP, uE_3, total hCG and inhibin-A	PAPP-A and free β-hCG AFP, uE_3 and inhibin-A	NT and PAPP-A AFP, uE_3, total hCG and inhibin-A	NT, PAPP-A and free β-hCG AFP, uE_3, and inhibin-A
55	0.4	0.4	0.1	0.1
60	0.6	0.6	0.1	0.1
65	0.9	1.0	0.2	0.2
70	1.4	1.5	0.3	0.3
75	2.1	2.3	0.5	0.6
80	3.2	3.5	0.9	1.0
85	5.1	5.6	1.7	1.9
90	8.8	9.5	3.4	3.7
95	17	18	8.2	8.8

Table 5a

False positive rate (%)	Detection Rate (%). Maternal age with: Preferred embodiment	Omitting inhibin-A	Omitting NT	Omitting NT and inhibin-A
1	81	76	66	60
3	89	86	79	74
5	92	90	85	80
7	94	92	88	84

Table 5b

Detection rate (%)	False-positive rate (%). Maternal age with: Preferred embodiment	Omitting inhibin-A	Omitting NT	Omitting NT and inhibin-A
60	0.1	0.2	0.6	1.0
70	0.3	0.5	1.4	2.2

80	0.9	1.5	3.2	5.0
90	3.4	5.0	8.8	12.6

Figs. 1 to 4 show the distributions of estimated risk of a term pregnancy with Down's syndrome in unaffected pregnancies and in Down's syndrome pregnancies using different markers in accordance with the present invention. In these figures, the vertical lines illustrate the detection rate (corresponding to the area under the Down's syndrome distribution curve to the right of the vertical line) achievable at a 5% false-positive rate (corresponding to the area under the unaffected distribution curve to the right of the vertical line). The dotted lines indicate uncertainties in the precise risk estimates.

Fig. 1 shows the distributions when using PAPP-A between 10 and 13 weeks and AFP, uE_3 and inhibin-A between 14 and 22 weeks.

Fig. 2 shows the distributions when using PAPP-A and free β-hCG between 10 and 13 weeks and AFP, uE_3 and inhibin-A between 14 and 22 weeks.

Fig. 3 shows the distributions when using NT and PAPP-A between 10 and 13 weeks and AFP, uE_3 inhibin A and total hCG between 14 and 22 weeks.

Fig. 4 shows the distributions when using NT, PAPP-A and free β-hCG between 10 and 13 weeks and AFP, uE_3 and inhibin-A between 14 and 22 weeks.

As an alternative, a sequential test can be performed. In this case the risk is initially determined based on only the marker levels from the first stage of pregnancy. This first estimate of risk is compared with a predetermined cut-off risk as is known for initial classification as screen-positive or screen-negative. Women having a screen-positive result are referred for a diagnostic test and might not be tested for screening marker levels at the second stage of pregnancy.

Women initially classified as screen-negative are

retested for markers measured at the second stage of pregnancy. The risk of Down's syndrome is determined again using one of two options.

As a first option, the markers from both the first and second stages of pregnancy are used. In determining the risk, the likelihood ratio can be calculated in the same way as in the non-sequential test described first above. Again it is desirable to take account of any correlation between the markers. As a refinement, the distributions of the screening markers used in determining the likelihood ratio arising from the combination of markers from the first and second stages of pregnancy are modified to allow for the fact that women with screen-positive results at the first stage of pregnancy have been removed from the sample presenting for the second test. The modified distributions of the markers measured at the second stage of pregnancy are typically derived by computer simulation.

The likelihood ratio computed in this way is then multiplied by the background risk, expressed as an odds ratio, after adjusting the background risk to take account of the fact that the women having a screen-positive result in the initial test at the first stage of pregnancy have been removed from the sample. The background risk can be reduced by multiplying the original background risk for the population by the complement of the overall detection rate of the test at the first stage of pregnancy, that is the detection rate expressed as a proportion. For more accuracy, one can allow for the fact that the detection rate and the false-positive rate of the test at the first stage of pregnancy vary with age. This is done by determining the detection rate and false-positive rate of the test carried out at the first stage of pregnancy for each age band (eg.a year of maternal age). The age-specific detection and false-positive rates are then used to determine the number of affected and unaffected pregnancies at each age in a simulated population presenting for the test at the second stage of pregnancy, and thereby the

age-specific prevalence of Down's syndrome among these women.

The adjusted background risk is multiplied by the likelihood ratio to calculate the risk estimate. The estimated risk is classified as screen-positive or screen-negative based on a comparison with a predetermined cut-off. The appropriate cut-off level is selected to achieve an acceptable overall detection-rate and false-positive rate, taking into account the detection rate and false-positive rate of the first test. The overall detection rate and false-positive rate, can be estimated in the usual way for different cut-off levels, but using the residual age distributions of women with screen-negative results from the first test.

As a second but less effective option, the risk of Down's syndrome is calculated using the markers measured at the second stage of pregnancy, without considering the levels of markers measured at the first stage of pregnancy, but otherwise allowing for the effect of screening at the first stage by adjusting the background risk as described above.

For women classified as screen-negative in the first test, the alternative, sequential method increases the discriminatory power over tests carried out at a single stage of pregnancy. Such improvement is not achieved for the women classified as screen-positive in the first test because the screening marker levels from the second stage of pregnancy are not used. Whilst the overall benefit of the sequential test is not as great as the benefit of the non-sequential combined test first described, the sequential test may be better psychologically for the patients who receive an immediate result at the first stage of pregnancy without waiting until the second stage of pregnancy.

Figs. 5 to 9 are flowcharts illustrating a specific method according to the present invention which is explained in detail below.

In the first trimester at around 8 to 13 weeks, or preferably around 10 to 13 weeks, an ultrasound scan is taken in step 1 and the nuchal translucency (NT) marker and the

crown-rump length (CRL) are measured and recorded in step 2. At the same stage, a blood sample is drawn in step 3, and the separated serum is refrigerated in step 4, whereupon no action is taken during a wait in step 5 until after a second sample is drawn in the second trimester. The ultrasound scan 1 and the blood sample 3 may be performed as alternatives or together depending whether it is desired to use ultrasound markers, biochemical markers or both.

In the second trimester at around 14 to 22 weeks, a second blood sample is drawn in step 6. Subsequently in step 7, the first and second samples are assayed for the respective biochemical markers selected.

The processing of the measurements taken in steps 2 and 7 is described below and illustrated in the blocks numbered 8 and above in Figs 5 to 8. This processing may be implemented in a data processing apparatus, most suitably an appropriately programmed computer. Thus the blocks numbered 8 and above also illustrate elements of the computer program or programming methods which performs the processing. In particular, the process blocks represent processing performed by the computer processor. The data entry blocks represent data entry processing which may be implemented by use of appropriate data entry fields shown on a display into which data may be entered from the computer's keyboard. The data item blocks represent data used by the program. The stored data blocks represent stored reference data which may be stored in the memory of the computer in files referenced by the computer program.

Data input means are used to input the concentrations (levels) of the serum markers in step 8 and the NT marker level and CRL measurement in step 9. If the levels from the first trimester are input immediately after measurement a message may be automatically generated and displayed at an appropriate time in the second trimester to remind the user that measurements from a second sample are due.

In step 10, each marker level is re-expressed as a

multiple of the median (MoM) level for unaffected pregnancies of the same gestational age and output as data item 11.

Step 10 is illustrated in more detail in Fig. 6. Stored data LMP 27 and scan 28 specific to respective methods of estimating gestational age are used to select an equation which estimates the expected median concentrations for different gestational ages for each marker in step 29. Data LMP 27 is specific to estimation of gestational age based on the first day of the last menstrual period. Data scan 28 is specific to estimation of gestational age from an ultrasound measure of the fetus, usually a BPD or a CRL. The equations selected based on stored data 27 or 88 may be simple linear equations or may be more complicated. For example, in the case of inhibin A in the second trimester. Since inhibin-A levels decline at the start of the second trimester, and start to rise again after 17 weeks' gestation, it is preferable to use a log-quadratic regression to calculate the median inhibin-A level at different gestational ages. The following equation is suggested in Watt HC, Wald NJ, Huttly WJ (1998); The pattern of maternal serum inhibin-A concentrations in the second trimester of pregnancy. Pregnat Diagn 18, 846-848:

$$\log_{10} I = k + 0.0001864 \times (a - 120)^2$$

where I is the inhibin-A concentration, a is the gestational age in days and the coefficient k is separately derived for each screening centre.

Based on an input in step 30 of the gestational age at the date of the sample, for each marker in step 31 the expected median levels in unaffected pregnancies of the same gestational age is calculated using the equation selected in step 29. In step 32, each marker level input in step 8 is divided by the expected median for that marker to output the MoM as data item 11.

In step 12, the NT marker is re-expressed as a MoM and output as data item 13. The specific calculation of step 12 is illustrated in Fig. 6 and corresponds to the MoM calculation for the biochemical markers, except that the CRL

measurement input in step 9 is used as the estimate of gestational age. Stored data 33 represents the NT medians for different CRL measurements, preferably as an equation.

There can be considerable systematic variation in nuchal translucency (NT) measurements from one ultrasonographer to another. Therefore, the stored data 33 may, optionally, represent NT medians which are ultrasonographer-specific in cases where it has been possible to base this data on sufficiently large numbers of measurements taken by individual ultrasonographers. In step 34 stored data 33 is used to calculate the expected median NT levels in unaffected pregnancies of the same CRL, i.e. the same age. In step 35, the NT measurement input in step 9 is divided by the expected median NT to give the NT MoM which is output as data item 13.

Optionally, the MoMs 11 for the biochemical markers may be adjusted in step 14 which is illustrated in detail in Fig. 7. Based on an input of any one or more of maternal weight, ethnic group, diabetic status and the number of fetuses in steps 36 to 39, respectively, stored weight adjustment equations 40, ethnic group adjustments 41, diabetes correction factors 42 and multiple birth correction factors 43 are used in step 44 to adjust the MoMs 11. The adjusted MoMs are output as data item 15.

In step 16, a multivariate Gaussian analysis of the MoM for all the markers from each stage of pregnancy is performed. For use in this analysis, distribution parameters 18 are selected in step 17 which is described in more detail in Fig. 8. For each marker the distribution parameters are stored as reference data 45 to 48 for different methods of estimating gestational age (LMP or scan) and based on whether or not the MoM has been adjusted for maternal weight. In step 49, the appropriate distribution parameters are selected and output as data item 18.

The multivariate Gaussian analysis 16 outputs a likelihood ratio as data item 19. This needs to be multiplied by a background risk to derive the estimated risk of Down's

syndrome. Whilst an overall population risk may be used, the present method uses age-specific risks calculated in step 20 which is described in more detail in Fig. 9. The gestational age of the sample input in step 50 (or 30) and the date of the sample input in step 51 are used to calculate the expected date of delivery (EDD) in step 52. The maternal date of birth is input in step 53 and is combined with the EDD to calculate the age at EDD as data item 56. This is used in the stored age-specific risk equation 57 to output the age-specific risk as data item 21. The likelihood ratio 19 and age-specific risk 21 are multiplied in step 22 to output the estimated risk of Down's syndrome as data item 23. The estimated risk 23 is compared with a predetermined cut-off in step 24 to produce a screen-positive result 25 when the risk is equal to or greater than the cut-off, or a screen-negative result 26 otherwise.

The apparatus may be arranged to provide estimates of the expected screening performance (i.e. the detection rate, false-positive rate and odds of being affected given a positive result), taking into account the age distribution of the screened population, the combination of screening markers used, the risk cut-off used, and other factors. The performance observed in practice can then be compared to the expected performance as an aid to monitoring.

The values of the stored data used in the method described above depends on which markers from the two stages of pregnancy are selected to be used. Appropriate data values for each marker are known, for example from the references.

CLAIMS

1. A method of determining whether a pregnant woman is at an increased risk of having a fetus with Down's syndrome, the method comprising the steps of:

measuring at least one screening marker level from a first stage of pregnancy by:

(i) assaying a sample obtained from the pregnant woman at said first stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said first stage of pregnancy;

measuring at least one screening marker level from a second stage of pregnancy by:

(i) assaying a sample obtained from the pregnant woman at said second stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said second stage of pregnancy; and

determining the risk of Down's syndrome using the measured screening marker levels from both the first and second stages of pregnancy.

2. A method according to claim 1, wherein the step of determining the risk of Down's syndrome comprises deriving the likelihood ratio of Down's syndrome using a multivariate analysis based on distribution parameters derived from a set of reference data.

3. A method according to claim 2, wherein said multivariate analysis is a multivariate Gaussian analysis.

4. A method according to claim 2 or 3, wherein the step of determining the risk of Down's syndrome further comprises

multiplying the likelihood ratio by an age-specific risk.

5. A method according to any one of the preceding claims, further comprising the step of re-expressing each measured screening marker level as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant woman.

6. A method according to any one of the preceding claims, wherein said first stage of pregnancy is in the first trimester of pregnancy and said second stage of pregnancy is in the second trimester of pregnancy.

7. A method according to any one of the preceding claims, wherein said first stage of pregnancy is between 8 and 13 weeks of pregnancy and said second stage of pregnancy is between 14 and 22 weeks of pregnancy.

8. A method according to any one of the preceding claims, wherein said step of measuring at least one screening marker level from a first stage of pregnancy includes assaying a serum sample obtained from the pregnant woman at said first stage of pregnancy for PAPP-A, free ß-hCG or both.

9. A method according to any one of the preceding claims, wherein said step of measuring at least one screening marker level from a second stage of pregnancy includes assaying a serum sample obtained from the pregnant woman at said second stage of pregnancy for AFP, uE_3, inhibin-A, free ß-hCG, free α-hCG, total hCG or any set thereof.

10. A method according to any one of the preceding claims, wherein said step of measuring at least one screening marker level from a second stage of pregnancy includes assaying a urine sample obtained from the pregnant woman at said second stage of pregnancy for ß-core hCG or total oestriol or both.

11. A method according to any one of the preceding claims, wherein said step of measuring at least one screening marker level from a first or a second stage of pregnancy includes assaying a sample of cells obtained from the pregnant woman at said first or second stage of pregnancy.

12. A method according to any one of the preceding claims, further comprising adjusting any or all of the screening marker levels to allow for one or more factors selected from the group of maternal race, maternal weight, multiple birth and diabetic status.

13. A method according to any one of the preceding claims, further comprising the steps of:

obtaining said sample from the pregnant woman at said first stage of pregnancy and/or taking said ultrasound scan at said first stage of pregnancy; and

obtaining said sample from the pregnant woman at said second stage of pregnancy and/or taking said ultrasound scan at said second stage of pregnancy.

14. A method according to any one of the claims 1 to 12, further comprising the steps of:

obtaining a first sample from the pregnant woman at said first stage of pregnancy;

storing the first sample under refrigeration; and

obtaining a second sample from the pregnant woman at said second stage of pregnancy,

wherein the first and second samples are assayed at the same time.

15. A method according to any one of the preceding claims, further comprising measuring at least one further screening marker level from at least one further stage of pregnancy and additionally using said at least one further screening marker in said step of determining the risk of Down's syndrome.

16. A method according to any one of preceding claims, further comprising:

determining a first risk estimate of Down's syndrome using the measured screening marker levels from the first stage of pregnancy;

comparing the first risk estimate with a predetermined cut-off level to initially classify the pregnant woman as screen-positive or screen-negative based on the comparison; and

performing said steps of measuring at least one screening marker level from a second stage of pregnancy and determining the risk of Down's syndrome using the measured screening marker levels from both the first and second stages of pregnancy if the pregnant woman is initially classified as screen-negative.

17. A method of determining whether a pregnant woman is at an increased risk of having a fetus with Down's syndrome, the method comprising the steps of:

measuring at least one screening marker level from a first stage of pregnancy by:

(i) assaying a sample obtained from the pregnant woman at said first stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said first stage of pregnancy;

determining a first risk estimate of Down's syndrome using the measured screening marker levels from the first stage of pregnancy;

comparing the first risk estimate with a predetermined cut-off level to initially classify the pregnant woman as screen-positive or screen-negative based on the comparison; and

if the pregnant woman is initially classified as screen-negative:

measuring at least one screening marker level from a second stage of pregnancy by:

(i) assaying a sample obtained from the pregnant woman at said second stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said second stage of pregnancy; and

determining the risk of Down's syndrome using the measured screening marker levels from second stage of pregnancy.

18. A method according to claim 16 or 17, wherein said step of determining the risk of Down's syndrome comprises calculating a likelihood ratio and multiplying by an age-specific risk which is adjusted, relative to the original age-specific risk of the original population presenting for the test at the first stage of pregnancy, to allow for fact that the proportion of affected pregnancies initially classified as screen-positive have been removed from the sample presenting for the test at the second stage of pregnancy.

19. A method according to claim 16 or 17, wherein said step of determining the risk of Down's syndrome comprises calculating a likelihood ratio and multiplying by an age-specific risk which is adjusted, relative to the original age-specific risk of the original population presenting for the test at the first stage of pregnancy, to allow for the fact that the age-specific proportions of affected and unaffected pregnancies initially classified as screen-positive have been removed from the sample presenting for the test at the second stage of the pregnancy.

20. A method according to any one of claims 16 to 19, wherein said step of determining the risk of Down's syndrome comprises calculating a likelihood ratio based on distributions of

markers which are modified, relative to the original distributions of markers of the original population presenting for the test at the first stage of pregnancy, to allow for the fact that women initially classified as screen-positive have been removed from the sample presenting for the test at the second stage of pregnancy.

21. A method according to any one of the preceding claims, further comprising comparing the determined risk with a predetermined cut-off level to classify the pregnant woman as screen-positive or screen negative based on the comparison.

22. A method according to claim 21 when dependent on any one of claims 16 to 20, wherein said second-mentioned cut-off level is determined based on the residual age distribution of pregnant women initially classified as screen-negative.

23. An apparatus for determining whether a pregnant woman is at an increased risk of having a fetus with Down's syndrome, the apparatus comprising:

data input means for inputting a measurement of at least one screening marker level from a first stage of pregnancy obtained by:

(i) assaying a sample obtained from the pregnant woman at said first stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said first stage of pregnancy;

data input means for inputting a measurement of at least one screening marker level from a second stage of pregnancy obtained by

(i) assaying a sample obtained from the pregnant woman at said second stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an

ultrasound scan taken at said second stage of pregnancy; and

calculation means for determining the risk of Down's syndrome using the input screening marker levels from both the first and second stages of pregnancy.

24. An apparatus according to claim 23, wherein said calculation means is arranged to determine the risk of Down's syndrome by deriving the likelihood ratio of Down's syndrome using a multivariate analysis based on distribution parameters derived from a set of reference data.

25. An apparatus according to claim 24, wherein said multivariate analysis is a multivariate Gaussian analysis.

26. An apparatus according to any one of claims 23 to 25, further comprising means for re-expressing each input screening marker level as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant woman and supplying the re-expressed screening marker level to said calculation means.

27. An apparatus according to any one of claims 25 to 28, further comprising data input means for inputting a measurement of at least one further screening marker level from at least one further stage of pregnancy and wherein said calculation means is arranged to determine the risk of Down's syndrome additionally using said at least one further screening marker level.

28. An apparatus according to any one of claims 23 to 27, further comprising means for comparing the determined risk with a predetermined cut-off level to classify the pregnant women as screen-positive or screen-negative based on the comparison.

29. A computer program which when executed on a computer causes the computer to perform a process for determining a pregnant woman's risk of having a fetus with Down's syndrome, the process comprising the steps of:

inputting a measurement of at least one screening marker level from a first stage of pregnancy obtained by:

(i) assaying a sample obtained from the pregnant woman at said first stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said first stage of pregnancy;

inputting a measurement of at least one screening marker level from a second stage of pregnancy obtained by

(i) assaying a sample obtained from the pregnant woman at said second stage of pregnancy for at least one biochemical screening marker; and/or

(ii) measuring at least one screening marker from an ultrasound scan taken at said second stage of pregnancy; and

determining the risk of Down's syndrome using the input screening marker levels from both the first and second stages of pregnancy.

30. A computer program according to claim 29, wherein said determination of the risk of Down's syndrome is performed by deriving the likelihood ratio of Down's syndrome using a multivariate analysis based on distribution parameters derived from a set of reference data.

31. A computer program according to claim 30, wherein said multivariate analysis is a multivariate Gaussian analysis.

32. A computer program according to any one of claims 29 to 31, wherein the process further comprises the step of re-expressing each input screening marker level as a multiple of

the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant woman, the re-expressed screening marker level being used in said determination of the risk of Down's syndrome.

33. A computer program according to any one of claims 29 to 32, wherein the process further comprises inputting a measurement of at least one further screening marker level from at least one further stage of pregnancy and wherein said determination of the risk of Down's syndrome additionally is performed using said at least one further screening marker level.

34. A computer program according to any one of claims 29 to 33, wherein the process further comprises the step of comparing the determined risk with a predetermined cut-off level to classify the pregnant woman as screen-positive or screen-negative based on the comparison.

35. A computer program recording medium storing a computer program according to any one of claims 29 to 34.

36. A method of determining whether a pregnant woman is at an increased risk of having a fetus with Down's syndrome substantially as hereinbefore described with reference to the accompanying drawings.

37. An apparatus for determining whether a pregnant woman is at an increased risk of having a fetus with Down's syndrome constructed and arranged to operate substantially as hereinbefore described with reference to the accompanying drawings.

FIG. 1

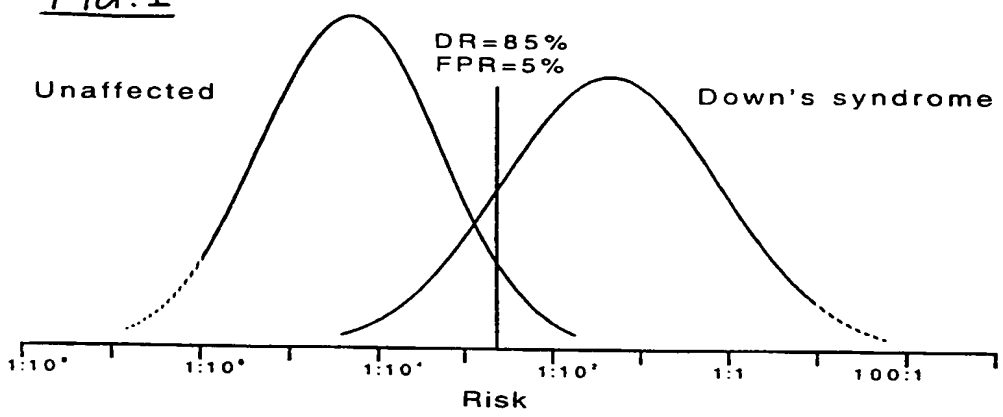


FIG. 2.

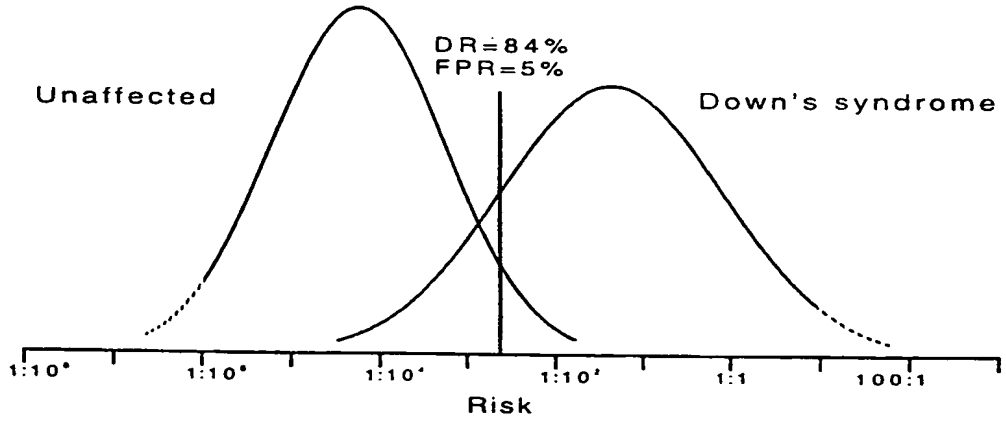


FIG.3

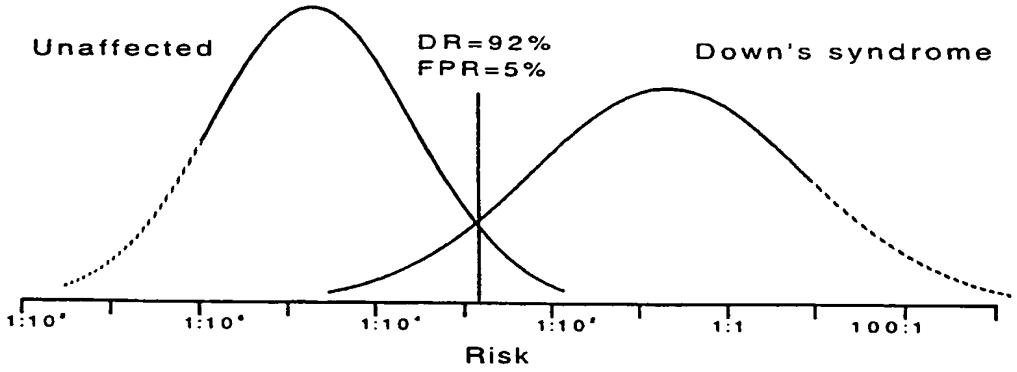


FIG.4

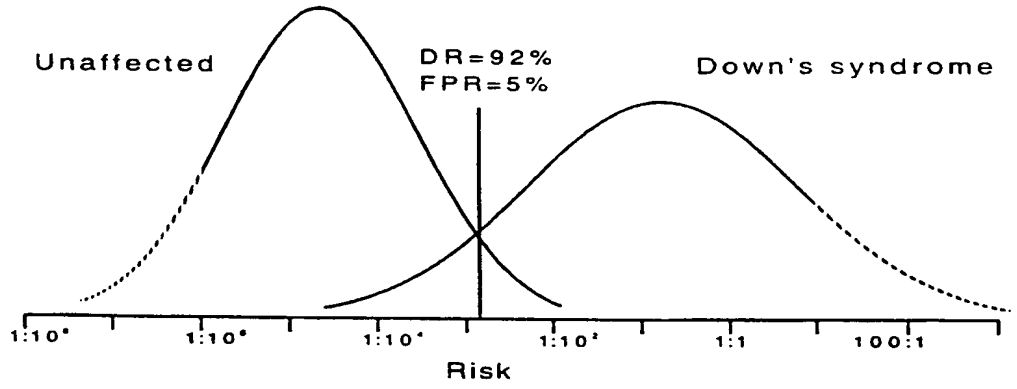


Figure 5

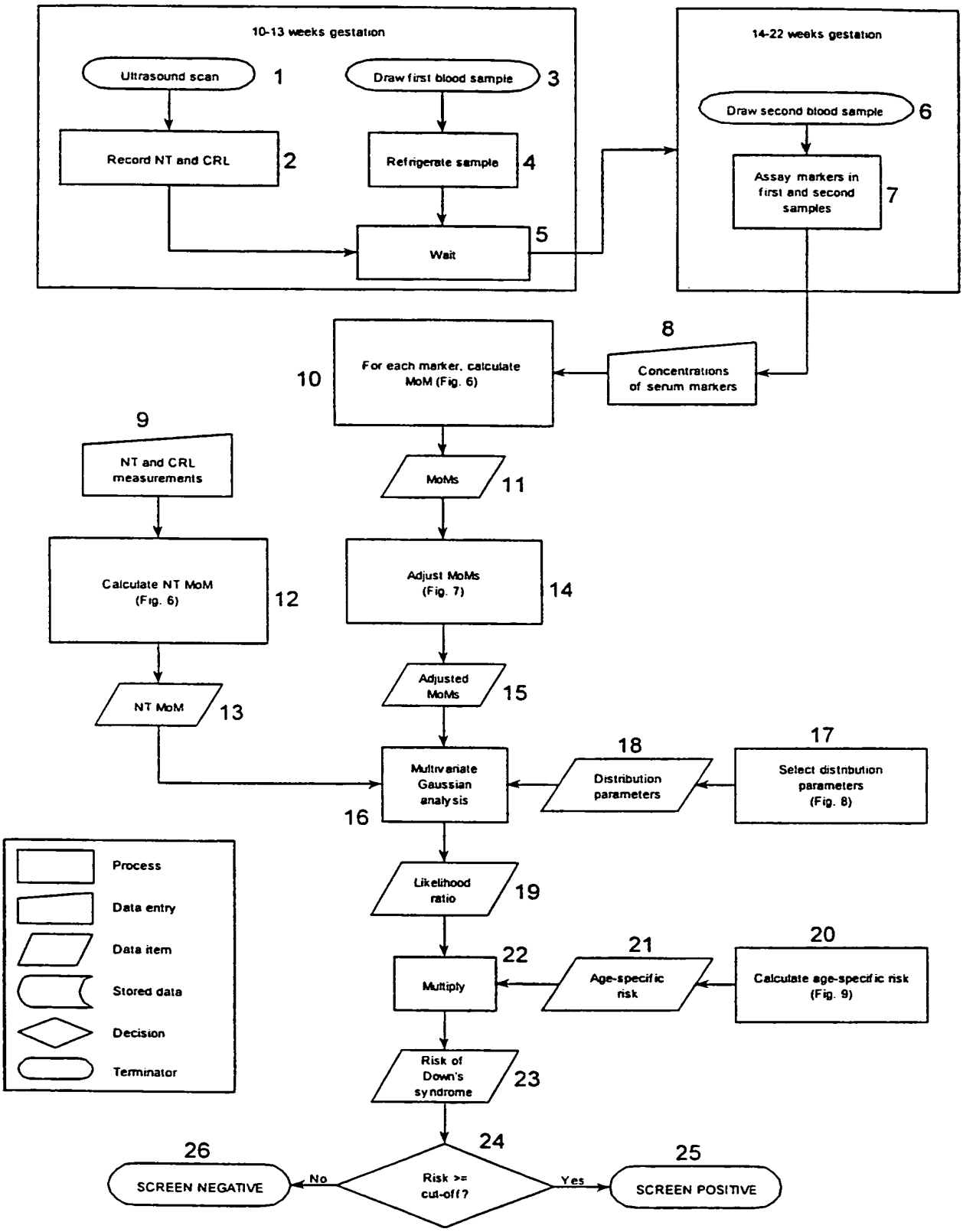


Figure 6

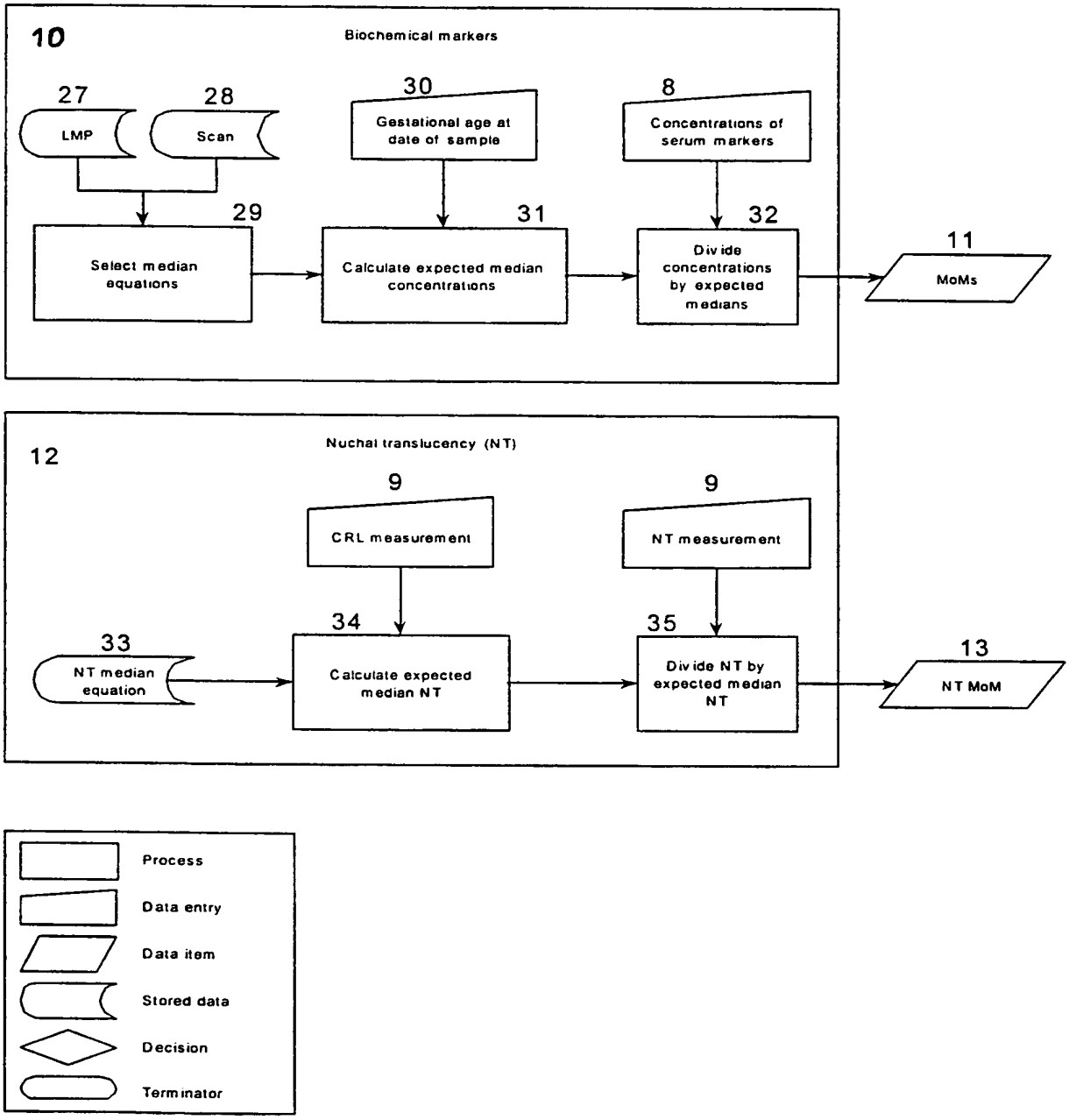


Figure 7

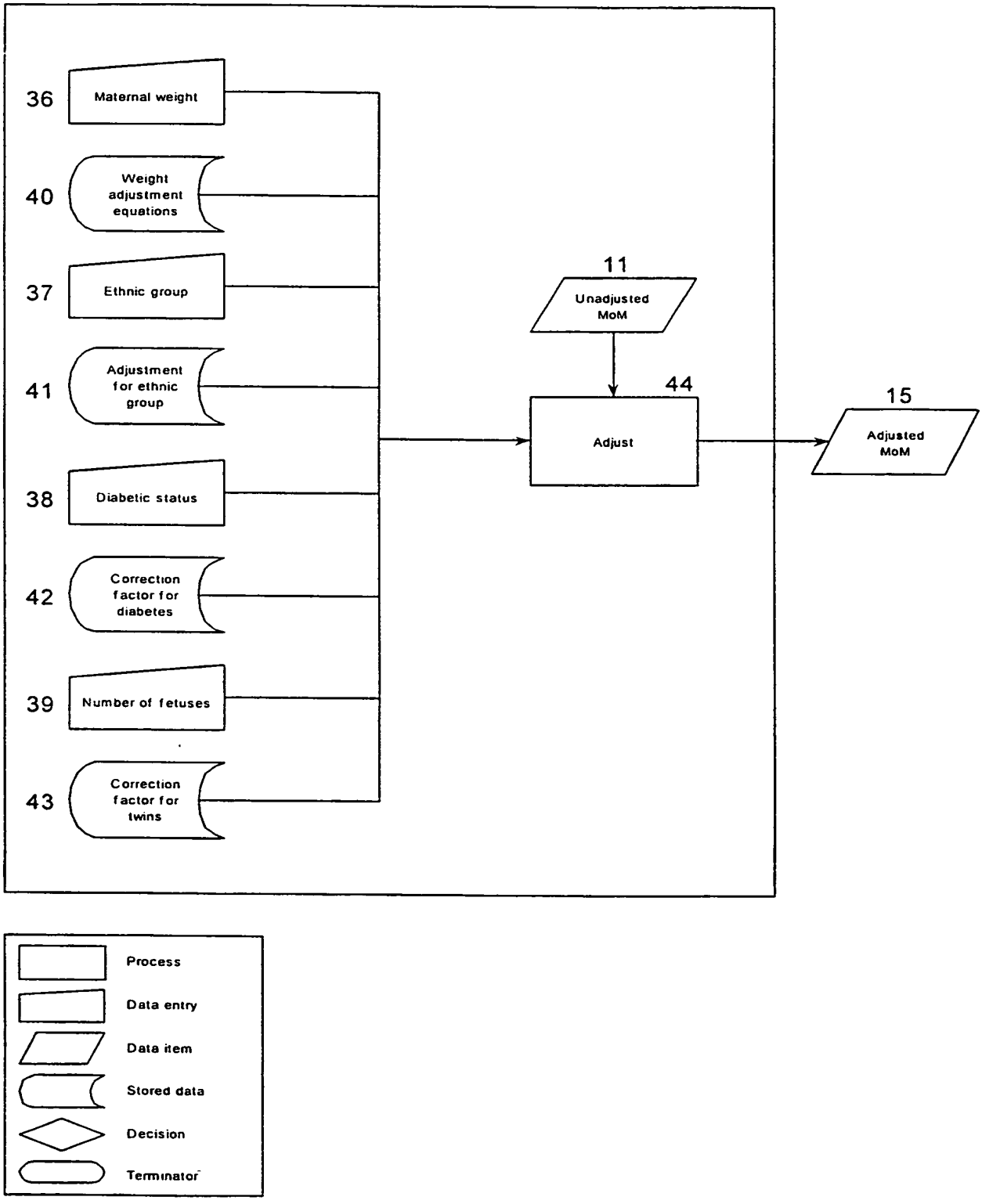


Figure 8

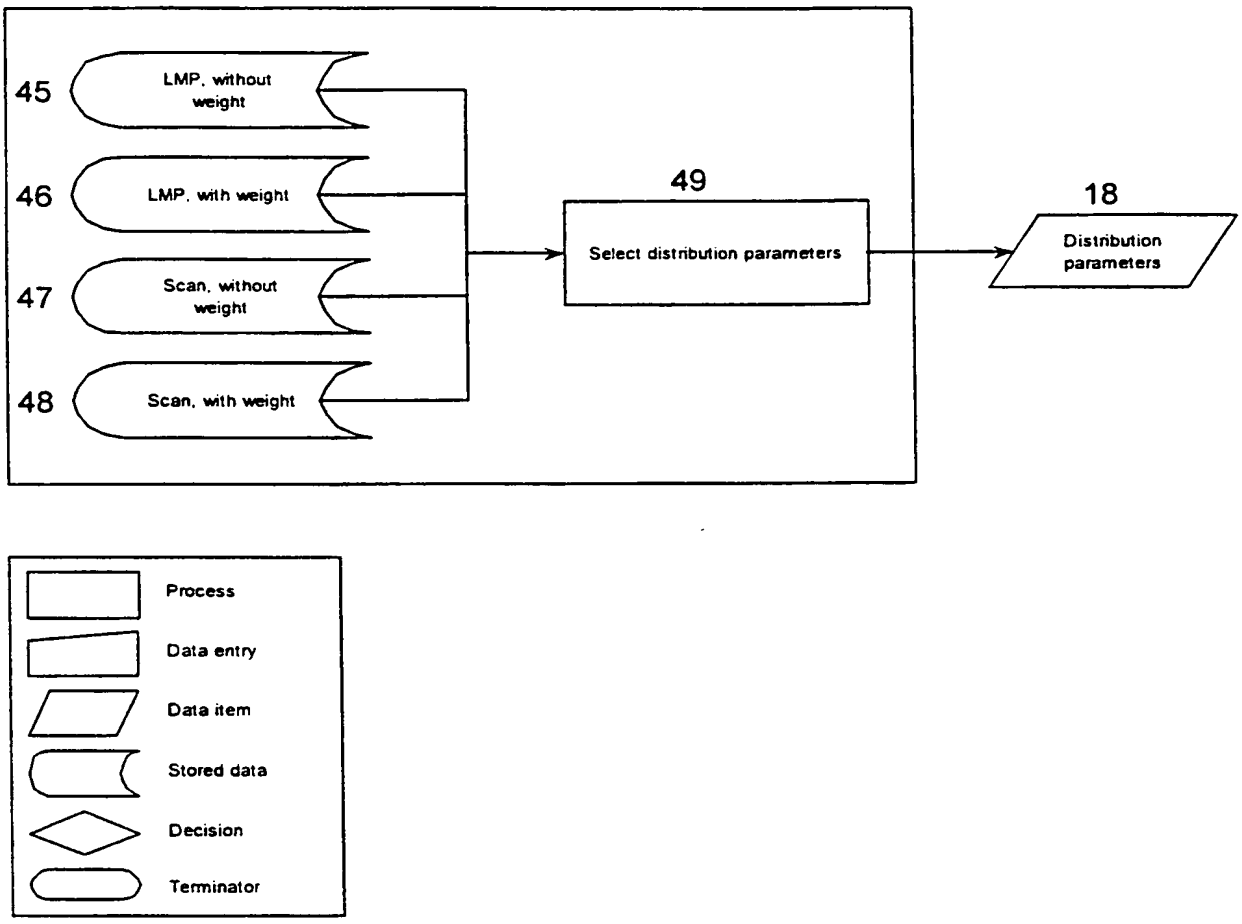
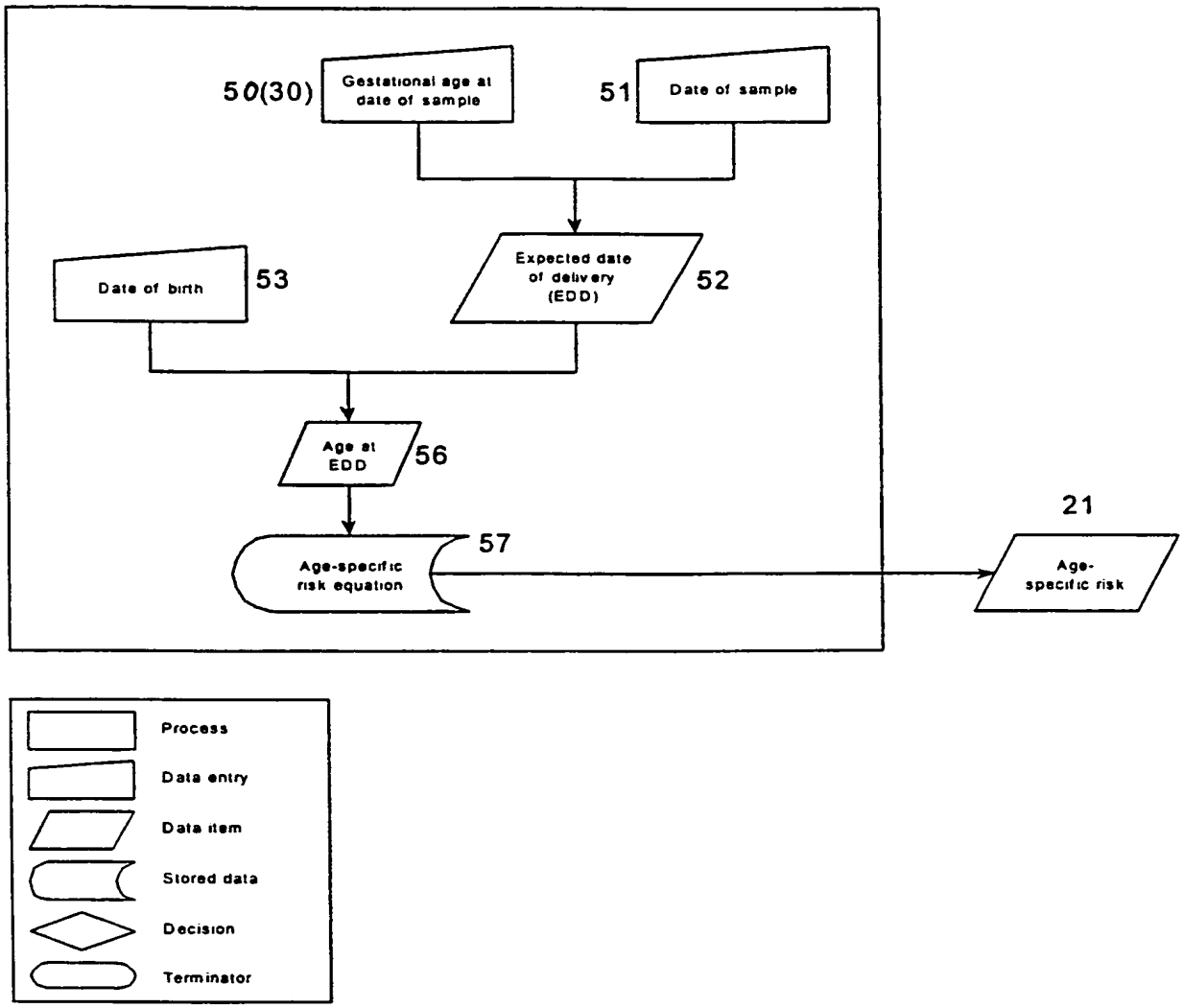


Figure 9



INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/GB 99/01341

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68 A61B8/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 03363 A (CIBA CORNING DIAGNOSTICS CORP ;CUCKLE HOWARD S (GB); CHARD TIMOTHY) 30 January 1997 (1997-01-30) claim 19 ---	1-28
A	WALD N J ET AL: "FIRST TRIMESTER BIOCHEMICAL SCREENING FOR DOWN'S SYNDROME" ANNALS OF MEDICINE, vol. 26, no. 1, 1 January 1994 (1994-01-01), pages 23-29, XP000672936 page 26, right-hand column, line 4 - line 25 -----	1-28

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 August 1999	20/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hoekstra, S

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Form PCT/ISA/210 (second sheet) (July 1992)

BNSDOCID: <WO___9956132A1_I_>

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB 99/01341

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 29-35
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(vi) PCT - Program for computers

2. [X] Claims Nos.: 36, 37
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos..

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Present claims 36 and 37 relate to a method and an apparatus defined by reference to a desirable characteristic or property, namely determing a fetus's risk of being affected by Down's syndrome.
These claims lack clarity (Article 6 PCT). An attempt is made to define the method and apparatus by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to technicall defined subject-matter limited to involving at least two consecutive measurements of a combimnation of at least one ultrasonographic marker and at least one biochemical marker.
Note that claims 36 and 37 do not meet the prescribed requirements of Rule 6.3(a) PCT.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 36, 37

Present claims 36 and 37 relate to a method and an apparatus defined by reference to a desirable characteristic or property, namely determing a fetus's risk of being affected by Down's syndrome.
These claims lack clarity (Article 6 PCT). An attempt is made to define the method and apparatus by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to technicall defined subject-matter limited to involving at least two consecutive measurements of a combimnation of at least one ultrasonographic marker and at least one biochemical marker.
Note that claims 36 and 37 do not meet the prescribed requirements of Rule 6.3(a) PCT.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/GB 99/01341

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9703363 A	30-01-1997	US 5716853 A	10-02-1998
		AU 6483096 A	10-02-1997